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REVIEW

State of the Art in the Culture of the Human Microbiota: New Interests and Strategies

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SUMMARY The last 5 years have seen a turning point in the study of the gut microbiota with a rebirth of culture-dependent approaches to study the gut microbiota. High-throughput methods have been developed to study bacterial diversity with culture conditions aimed at mimicking the gut environment by using rich media such as YCFA (yeast extract, casein hydrolysate, fatty acids) and Gifu anaerobic

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medium in an anaerobic workstation, as well as media enriched with rumen and blood and coculture, to mimic the symbiosis of the gut microbiota. Other culture conditions target phenotypic and metabolic features of bacterial species to facilitate their isolation. Preexisting technologies such as next-generation sequencing and flow cytometry have also been utilized to develop innovative methods to isolate previously uncultured bacteria or explore viability in samples of interest. These techniques have been applied to isolate CPR (Candidate Phyla Radiation) among other, more classic approaches. Methanogenic archaeal and fungal cultures present different challenges than bacterial cultures. Efforts to improve the available systems to grow archaea have been successful through coculture systems. For fungi that are more easily isolated from the human microbiota, the challenge resides in the identification of the isolates, which has been approached by applying matrix-assisted laser desorption ionization–time of flight mass spectrometry technology to fungi. Bacteriotherapy represents a nonnegligible avenue in the future of medicine to correct dysbiosis and improve health or response to therapy. Although great strides have been achieved in the last 5 years, efforts in bacterial culture need to be sustained to continue deciphering the dark matter of metagenomics, particularly CPR, and extend these methods to archaea and fungi.

KEYWORDS culture strategies, human microbiota, bacteria, archaea, fungi, culturomics

INTRODUCTION

Next-generation sequencing has become the gold standard for studies exploring the diversity of the gut microbiota (1). This method has led to uncovering how instrumental the human microbiota is for homeostasis and how negatively impactful a dysbiotic microbiota can be for human health even in the early stages of life (2, 3). This wealth of knowledge and new understanding has also preempted a rebirth of culture techniques to explore the gut microbiota (4, 5) to respond to the need of strains for further experimentation. In 2015, we reviewed the past and current strategies for bacterial culture and illustrated the rebirth of culture methods exemplified through "microbial culturomics" (4, 5). We also highlighted the need to develop new strategies to obtain gut microbiota isolates, specifically anaerobic strains, that were dramatically under isolated. Here, we review the advances in strategies in the last 5 years to study the human microbiota, namely, bacteria, archaea, and eukaryotes. A table of the culture media mentioned in this review is also provided (see Table 1 below).

NEW STRATEGIES TO ISOLATE BACTERIA FROM HUMAN MICROBIOTA

Background

The advent of next-generation sequencing (NGS) has allowed a better exploration and therefore a better understanding of the human microbiome, mostly through amplicon sequencing and whole-genome metagenomics. Indeed, NGS, which allows relatively fast analysis, has quickly replaced standard culture techniques that are very fastidious. NGS has allowed large data sets to be generated and linked dysbiosis to an increasing number of ailments. However, NGS presents several limitations, such as the lack of standardization with the variability of extraction protocols and primers, the depth bias associated with all molecular methods, causing minority species to be overlooked, the lack of information about viability, and the availability of strains for further experimentation (1, 4, 6). All of these limitations highlight the necessity of a return to culture-dependent methods complemented with culture-independent methods. The culturomics concept, which consists of high-throughput culture, was created in 2012 for this purpose (7). Since then, many studies have combined high-throughput culture with metagenomics and reduced the dark matter associated with metagenomics through the isolation and sequencing of previously uncultured species. In fact, Lagier et al. compared genomic sequences obtained from new species identified in previous studies with the reads found in the Human Microbiome Project (8). A total of 50.6% of the operational taxonomic units (OTUs) in these new sequences, including

TABLE 1 Media mentioned in this review and their applications

Media	Abbreviation	Additional ingredients	Favored microorganism	Reference(s)
Bacteria				
Yeast extract-casein hydrolysate-fatty acids	YCFA	—[a]	Aero-intolerant bacteria	49, 56
		Defibrinated sheep blood (5%), rumen fluid (5%), and LpxC enzyme (CHIR-090) (400 μg/ml)	Gram-positive aero-intolerant bacteria	49
		Glucose (0.002 g/ml), maltose (0.002 g/ml), and cellobiose (0.002 g/ml)	Gut aero-intolerant bacteria	18
		Glucose (0.002 g/ml), maltose (0.002 g/ml), cellobiose (0.002 g/ml), and sodium taurocholate (0.1%)	Spore-forming gut aero-intolerant bacteria	18
Gifi anaerobic medium	GAM	—	Aero-intolerant bacteria	21, 34, 58
		Glucose (1%)	Aero-intolerant bacteria	51
		Starch, peptone, yeast extract, meat extract, liver digest, serum pancreatic digest, L-tryptophan, L-arginine, L-cysteine, vitamin K_1, hemin, sodium chloride, and sodium thioglycolate	Aero-intolerant bacteria	58
Bicarbonate buffered anoxic medium		—	Aero-intolerant bacteria	34
		Tobramycin (25 μg/ml), polymyxin E (10 μg/ml), and cefotaxime (10 μg/ml)	Aero-intolerant bacteria	34
		Fecal supernatant (1%)	Aero-intolerant bacteria	34
Schaedler agar modified (R-medium)		Ascorbic acid[b], glutathione[b], uric acid[b], α-ketoglutarate, and hemin	Aero-intolerant bacteria	47
Wilkins Chalgren anaerobe agar		—	Aero-intolerant bacteria	39, 40
		Mupirocin (100 mg/liter), glacial acetic acid (1 ml/liter), norfloxacin (200 mg/liter), and 8-hydroxyquinoline (90 mg/liter)	*Bifidobacterium* spp.	39
Modified Wilkins-Chalgren agar		Soya peptone (5 g/liter), L-cysteine (0.5 g/liter), Tween 80 (1 ml/liter), mupirocin (100 mg/liter), and glacial acetic acid (1 ml/liter)	*Bifidobacterium* spp.	40
Brain heart infusion	BHI	—	Rich conventional medium	10, 58
Brain heart infusion agar	BHI-Agar	Defibrinated sheep blood (10%)	Rich conventional medium	10
Supplemented brain heart infusion	BHIych	Yeast extract (5 g/liter), cysteine (1 g/liter), and hemin (15 mg/liter)	Gut aero-intolerant bacteria	56
	LYHBHI	Yeast extract (5 g/liter), cysteine (1 g/liter), hemin (15 mg/liter), cellobiose (1 g/liter), and maltose (1 g/liter)	Gut aero-intolerant bacteria	56
		Various additional factors[c]	CPR	10
Gut microbiota medium	GMM	—	Gut aero-intolerant bacteria	58
Fastidious anaerobe agar	FAA	Defibrinated horse blood (5%)	Aero-intolerant bacteria	56, 78
Fastidious anaerobe broth	FAB	—	Aero-intolerant bacteria	78
		Kanamycin (100 μg/ml)	CPR	78
T-Raoult medium		—	*Treponema* spp.	31
Oral treponeme enrichment broth	OTEB	—	*Treponema* spp.	10
		Various additional factors[c]	CPR	10
MTGE-anaerobic enrichment broth	MTGE	—	Aero-intolerant bacteria	10
		Various additional factors[c]	CPR	10
Defined mucin medium	DMM	—	Oral bacteria	10
		Glucose (2%), Casamino Acids (0.1 g/liter), and defibrinated sheep blood (10%)	CPR	10
R2A broth medium	R2A	—	Poor nonselective medium	36
		Vancomycin (300 μg/ml) and amphotericin B (5 μg/ml)	Gram-negative bacteria from human skin	36
Trypticase peptone		—	Poor nonselective medium	51
Hanks balanced salt solution	HBSS	—	Poor nonselective medium	36
		Vancomycin (300 μg/ml) and amphotericin B (5 μg/ml)	Gram-negative bacteria from human skin	36
Tryptic soy broth	TSB	—	Rich nonselective medium	10, 51
		Various additional factors[c]	CPR	10
SHI medium	SHI	—	Oral bacteria	81
		Streptomycin (100 to 500 μg/ml)	CPR	81
Mueller Hinton	MH	—	Rich nonselective medium	58
BY-chocolate agar	Choco	—	Nonselective medium	58
Mannitol salt agar	MSA	—	Halotolerant bacteria	58
Peptone–yeast extract broth	PY	1% glucose	Phenol- and *p*-cresol-producing bacteria	51
Phenylethyl alcohol agar	PEA	Defibrinated sheep blood (5%)	Gram-positive aerotolerant bacteria	58
Columbia colistin nalidixic acid agar	CNA	Defibrinated sheep blood (5%)	Gram-positive aerotolerant bacteria	58
DeMan, Rogosa, and Sharpe broth	MRS	—	*Lactobacillus* spp.	51, 58

(Continued on next page)

TABLE 1 (Continued)

Media	Abbreviation	Additional ingredients	Favored microorganism	Reference(s)
Tomato juice agar	Tomato	–	*Lactobacillus* spp.	58
Mitis *Salivarius* agar	MS Agar	–	*Streptococcus* spp. and *Enterococcus* spp.	58
KF *Streptococcus* agar	KF	–	*Streptococcus* spp.	58
Cycloserine cefoxitin fructose agar	CCFA	–	*Clostridium difficile*	58
Listeria enrichment broth	LEB	–	*Listeria* spp.	58
Drigalski lactose agar	BTB	–	Gram-negative bacteria	58
Deoxycholate hydrogen sulfide lactose agar	DHL	–	Gram-negative bacteria	58
Bacteroides bile esculin	BBE	–	Aero-intolerant gram-negative bacteria	58
Selenite broth	Selenite	–	*Salmonella* spp.	58
Tetrathionate broth	TT	–	*Salmonella* spp.	58
FM agar, modified	FM	–	*Fusobacterium* spp.	58
Cefsulodin-Irgasan-novobiocin agar	CIN	–	*Yersinia enterocolitica*	58
Thayer-Martin selective agar	TM	–	*Neisseria* spp.	58
Skirrow's medium	Skirrow	–	*Campylobacter* spp.	58
Thiosulfate citrate biliary salt agar	TCBS	–	*Vibrio* spp.	58
Archaea				
SAB medium	SAB	–	Methanogenic archaea	92
Eukaryotes				
Potato dextrose agar	PDA	–	Yeasts and filamentous fungi	58, 123, 127
		Yeast extract, colistin (30 mg/liter), vancomycin (30 mg/liter), and imipenem (30 mg/liter)	Yeasts and filamentous fungi	123
Sabouraud dextrose broth	SDB	–	Yeasts and filamentous fungi	123, 124
		Defibrinated sheep blood (5%) and rumen juice (5%)	Yeasts and filamentous fungi	123
		Defibrinated human blood (7%) and glucose (3%)	Yeasts and filamentous fungi	124
Sabouraud dextrose agar	SDA	–	Yeasts and filamentous fungi	58, 123, 124, 127
		Colistin (30 mg/liter), vancomycin (30 mg/liter), and imipenem (30 mg/liter)	Yeasts and filamentous fungi	123
		Chloramphenicol (40 μg/ml) and kanamycin (50 μg/ml)	Yeasts and filamentous fungi	124, 127
Czapek-Dox agar	CZAPEK	–	Yeasts and filamentous fungi	127
		Chloramphenicol (50 μg/ml) and gentamycin (100 μg/ml)	Yeasts and filamentous fungi	127
Schaedler agar		–	Yeasts and filamentous fungi	123
		Malt extract, ox bile, oleic acid, glycerol, Tween 60, colistin (30 mg/liter), vancomycin (30 mg/liter), and imipenem (30 mg/liter)	Yeasts and filamentous fungi	123
Banana agar medium	BAM	Colistin (30 mg/liter), vancomycin (30 mg/liter), and imipenem (30 mg/liter)	Yeasts and filamentous fungi	123
Glycine-vancomycin-polymyxin B agar		–	Yeasts and filamentous fungi	125
Malt agar		–	Yeasts and filamentous fungi	125
Dixon agar	DIX	–	Lipophilic yeast	123, 127
		Colistin (30 mg/liter), vancomycin (30 mg/liter), and imipenem (30 mg/liter)	Lipophilic yeast	123
		Chloramphenicol (50 μg/ml) and cycloheximide (200 μg/ml)	Lipophilic yeast	127
Modified Leeming and Notman agar	mLNA	–	Lipophilic yeast	126
		Chloramphenicol (50 μg/ml) and streptomycin (100 μg/ml)	Lipophilic yeast	126
CHROMagar Candida		–	Yeast	124

[a]–, No added ingredient.
[b]Antioxidant.
[c]ATCC vitamin, trace minerals, clarified filtered saliva, pig gastric mucin, sugars, amino-acids, nucleobases, *N*-acetyl muramic acid, *N*-acetylglucosamine, pyruvate.

high-abundance OTUs, matched OTUs from the HMP reference list and allowed the generation of a large number of previously unknown genes and associated functions (ORFans). Other teams have also been able to match new species with unassigned OTUs (9, 10). Consequently, efforts in the shift toward culture to study the human microbiota need to be sustained. Here, we review the new strategies developed to improve the isolation of bacteria from the human microbiota.

Sampling and Conservation for Culturing the Human Microbiota

Sampling is an essential step in the study of the human microbiota. Past and current

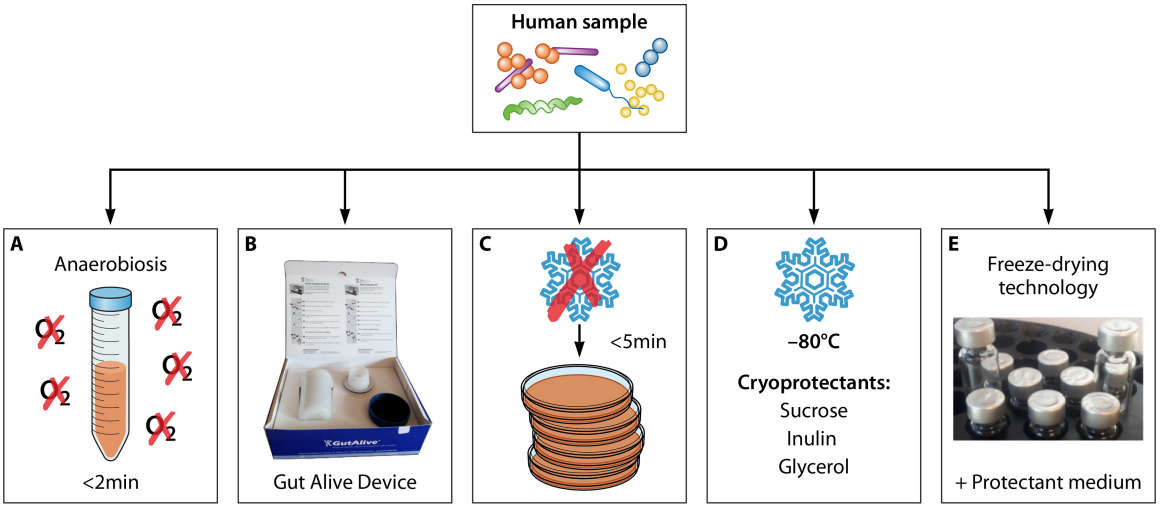

FIG 1 Yield increasing storage of samples. Conditions of collection and storage of human clinical samples may affect the viability of microorganisms. (A) Short exposure to oxygen of the interest sample increases viability (17). (B) Commercially available sampling kits were available and had been shown to be effective on the viability of fastidious microorganisms (12). (Reprinted from reference 12.) (C) The better alternative remains culture of the sample prior to cryopreservation (15). (D and E) Human clinical specimens are mainly stored using cryopreservation or freeze-drying; the addition of cryoprotectants or a protectant medium allows for a better yield after thawing (15, 16).

studies have shown that the method of conservation greatly impacts the results obtained through culture-dependent methods (11). Freezing at −20°C or −80°C is used for sample storage before experimentation. Freezing at −80°C, often used for long-term storage, is considered the gold standard of sample conservation with special regard to the cold chain (12). For short-term storage, refrigeration (4°C) can be sufficient as opposed to storage at room temperature (13). Samples can also be stored after freeze-drying, which is used in some instances for fecal microbiota transplantation (14). Nevertheless, freeze-drying can be a destructive process for cells and greatly affects the viability of samples. The addition of protective agents such as disaccharides, polyols, and proteins separately (15) or, as shown in a recent study, a combination of all the above (16), has been described to improve the viability of bacteria in feces after freeze-drying.

Exposure to oxygen, even for 2 h, dramatically lowers the viability of a sample, as shown by our team, using "live/dead" staining and flow cytometry (11, 17). In fact, limiting the exposure to oxygen by shortening the time between sampling and culture (17) or using a GutAlive device or a preservation medium, facilitates the isolation of fastidious species (12, 13). The improvement of storage conditions and sampling can therefore allow the cultivation of fastidious and extremely oxygen-sensitive bacteria, such as *Faecalibacterium prausnitzii*, and improve the yield from a human sample (Fig. 1).

Recent Strategies To Isolate Bacteria from Human Microbiota

Using high-yield media. (i) Use of YCFA medium. Yeast extract–casein hydrolysate–fatty acid medium (YCFA) is a rich medium composed of growth factors, antioxidants, volatile fatty acids, and vitamins (https://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium1611.pdf). Since the human intestinal microbiota consists largely of oxygen-intolerant bacterial species, YCFA medium should be particularly suitable for the culture of this microbiota. A study by Browne et al. showed that a large proportion of the bacteria in the human intestinal microbiota can be cultured using this single medium. In this study, 137 species were isolated, among which were 68 new species (63 *Firmicutes*, 4 *Bacteroidetes*, and 1 *Actinobacteria*) (see Table S1 in the supplemental material) (18). Another study used direct inoculation of samples in YCFA medium, leading to the isolation of 273 different bacterial species, including 105 new bacterial species (9) distributed into 3 phyla (91 *Firmicutes*, 13 *Bacteroidetes*, and 1

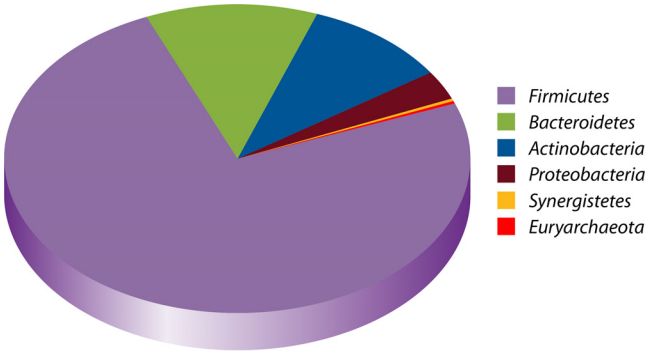

FIG 2 Phylogenic distribution of the new species isolated using high-throughput culture approaches. A total of 448 species were isolated in several projects from different teams using the culturomics approach or a culturomics-like approach. Six phyla are represented: *Firmicutes* (74.55%), *Bacteroidetes* (11.83%), *Actinobacteria* (9.82%), *Proteobacteria* (3.13%), *Synergistetes* (0.22%), and *Euryarchaeota* (0.22%).

Proteobacteria) (see Table S1). Other research teams used YCFA medium in their work as well, confirming its role in human microbial culture. The use of this single medium allowed (9, 18, 19) the expansion of the known microbiota through the discovery of numerous previously uncultured bacteria (Fig. 2).

(ii) Cultivation of dominant bacteria of the human intestinal microbiota using Gifu anaerobic medium. The "human microbial catalogue" established a list of gut-dominant species based on a common core of taxa found in 124 metagenomes from European individuals (20). A variety of media were recommended for the culture of 56 dominant gut bacteria (consisting at 96% of aero-intolerant species), among which only 6 were recommended to be grown on GAM. Gotoh et al. suggested a method to optimize the culture of dominant human gut bacteria using Gifu anaerobic medium (GAM) (21). A total of 32 dominant human gut species, including 17 *Firmicutes*, 14 *Bacteroidetes*, and 1 *Actinobacteria*, were able to grow on GAM by dissolving the oxygen generated by autoclave sterilization, thus showing that this medium can be suitable when studying dominant gut species.

(iii) Culture enrichment. Analysis of the original conditions used in culturomics highlighted prolific culture conditions. High-yielding culture conditions included enrichment of samples in blood culture bottles with and without supplementation with rumen fluid and/or sheep blood (8). Other studies have shown the importance of culture enrichment (19, 22). The combination of culture enrichment with supplementation with fresh medium led to an increase of 22% of species isolated (19). Moreover, culture enrichment improved sequencing depth for amplicons, as well as metagenomics (22).

Targeting phenotypic traits of bacterial species from human microbiota. (i) Improvement of subculture: experienced picking versus all picking. Recently, an optimization study of the culturomics method showed that the subculture strategy of colonies is likely to influence the number of species isolated from samples. Indeed, two picking methods were implemented: the "experienced picking" technique, picking only two to three colonies per plate, and the "picking all" technique, picking all the colonies on a plate. Only 8.5% fewer species were isolated through the experienced picking method compared to the picking all method, which requires a much heavier workload (19). Therefore, the experience of the investigator is critical when using culture-dependent methods to study the gut microbiota.

(ii) Culture of spore-forming bacteria, including *Clostridium difficile*. Spore-forming bacteria have a significant impact on human health. Using culture-independent techniques, it has been shown that approximately 50% of the healthy human intestinal microbiota are spore-producing bacteria, the majority of which are still uncultivated. Spore-forming bacteria have also been linked to dysbiotic conditions, and the implication of *C. difficile* in nosocomial infectious diarrhea and *Clostridium*

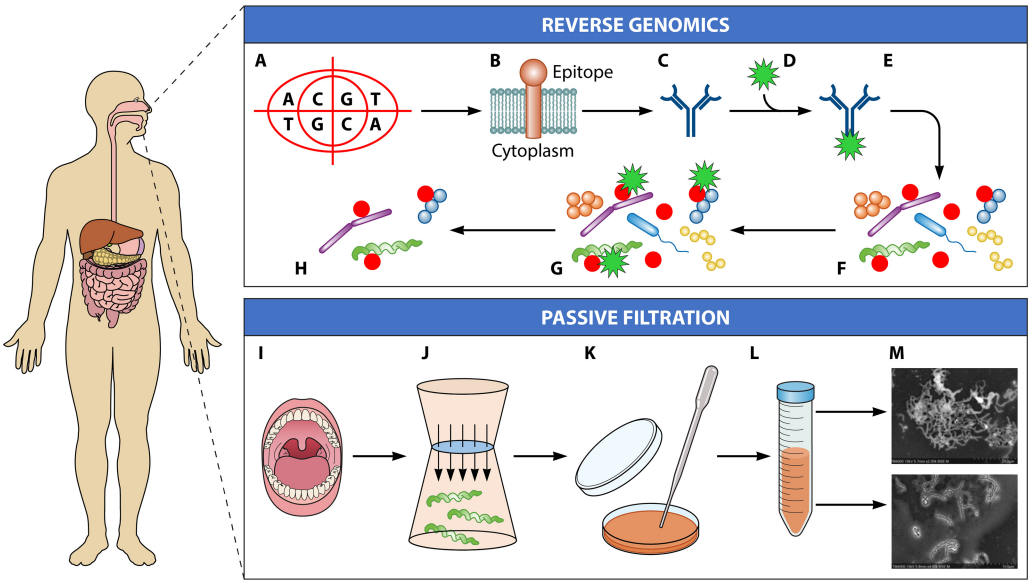

FIG 3 Innovative methods to isolate fastidious species. For microbial culture using the reverse genomic method, genes coding for membrane proteins were identified (A), allowing the selection of extracellular epitopes (B). Specific antibodies against these epitopes were produced (C), purified, and fluorescence labeled (D and E). Samples were stained using the designed antibodies (F), and the target cells were sorted using flow cytometry (G). (H) The sorted fractions were cultured to isolate the targeted microorganisms. *Spirochaetes* culture was recently innovated using passive filtration at 0.22 μm. Oral samples (I) were cultured in an anaerobic workstation in a nutrient medium (T-Raoult) (J). The lower compartment of the double chamber filter was inoculated on solid T-Raoult medium and incubated for 5 days at 37°C under anaerobic conditions. The colonies were then subcultured using a Pasteur pipette (K) and cultured in liquid medium (L and M).

butyricum in necrotizing enterocolitis is no longer to be demonstrated. This emphasizes the need to cultivate spore-forming bacteria from the human microbiota. Spore-forming species were historically isolated using heat shock or ethanol treatment (23). In fact, the culturomics method involves heat shocking the fecal sample for 20 min at 80°C to selectively isolate spore-forming species (4). Moreover, a significant study described improved culture conditions to allow targeted isolation of spore-forming bacteria by incubating the sample at equal volume of 70% alcohol for 4 h at room temperature in aerobic conditions prior to washing and plating (18). Browne et al. cultured spore-forming bacteria resistant to ethanol, including *C. difficile* (18); 66 resistant bacterial species, including 2 new families of bacteria, were subsequently identified. Similarly, a very recent study (24) isolated 60 species through ethanol treatment that were not isolated under the 22 other culture conditions used, including 9 new species (see Table S1). Primary and secondary bile acids, particularly taurocholate, have been shown to be potent germinants to facilitate the restoration of vegetative growth and metabolism (25, 26).

(iii) Using motility of *Treponema* for culture by passive filtration. The cultivation of *Spirochaetes* remains relatively difficult and fastidious, and very few studies on the subject have been carried out recently (27, 28). However, its involvement in human health is significant. For example, *Treponema pallidum* is the causative agent of syphilis in humans (29). The motility and small size of *Treponema* species have been previously exploited to improve their isolation through successive filtration from 5 to 0.22 μm (30). Similarly, passive filtration at 0.22 μm in culture enrichment steps (Fig. 3) led to the isolation of 5 strains of *Treponema denticola* and 10 strains of *Treponema pectinovorum* from the human oral cavity (31), thus providing a non-negligible improvement in the culture of treponemes.

(iv) Antibacterial activity of antibiotics or phages utilized for bacterial culture. Bacterial resistance to antibiotics is an emerging and major public health issue as well as a major topic in research. Studies have shown that the gut harbors antibiotic resistance genes probably acquired through lateral transfer from human pathogens (32,

33). Nevertheless, in bacterial culture, antibiotic resistance can be utilized as a tool to isolate previously uncultured species. For example, fecal samples from patients in intensive care, whose gut was selectively decontaminated using prophylactic antibiotherapy, were inoculated on a porous aluminum oxide chip and then deposited on a rich medium (GAM) and a poor medium (bicarbonate buffered anoxic medium) supplemented or not with antibiotics (34). 16S amplicon sequencing was used to identify the cultures. Using the location of the chip, unassigned OTUs were isolated in pure culture, and a new strain resistant to both metronidazole and imipenem was identified (34). Antibiotics can also be used to selectively culture nondominant species. For example, the proportion of Gram-negative bacteria has long been neglected from the human skin microbiota. Nevertheless, due to NGS, their presence in large proportions was highlighted (35). Using two different media to eliminate both Gram-positive bacteria and fungi, Hanks' balanced salt solution (HBSS) and R2A broth supplemented with vancomycin (300 μg/ml) and amphotericin B (5 μg/ml), Gram-negative bacteria were isolated from human skin samples, confirming their presence in the skin microbiota (36). Finally, using antibiotics, antagonism between species can be bypassed, as in the case of the antagonism between *Clostridia* and *Bifidobacteria* (37). In fact, in the past few years, scientists have been particularly interested in the cultivation of *Bifidobacteria* due the probiotic capacity of many species belonging to this genus (38). For that purpose, Novakova et al. used Wilkins Chalgren agar medium with mucin supplemented with a selective anticlostridial agent called 8-hydroxyquinoline to enhance the isolation of *Bifidobacteria* (39). Similarly, another study focused on the selection of *Bifidobacteria* using antibiotics and a new medium containing mupirocin, glacial acetic acid and norfloxacin (40). Bacterial growth can also be inhibited through the use of phages, decreasing the potential development of resistance associated with the use of antibiotics (41). Phages have previously been successfully used in culturomics studies (7). Since then, new phages have been isolated with, in some instances, activity against a broader range of species (42–45) and, in other instances, trained to gain a particular activity (41). Although phage therapy is the main goal, these phages can also be used to isolate previously uncultured species, as seen in previous studies (46).

Targeting metabolism to isolate bacterial species from human microbiota. It is a well-known fact that most bacteria are still uncultured. One main reason justifying this high proportion of uncultivated bacteria is the inability to mimic their natural habitat *in vitro*. The increased knowledge acquired through NGS allows a prediction of the metabolic capacities of sequenced bacterial species. As a result, this improved understanding of their metabolism can be used in service of their isolation.

(i) Enhancing anaerobiosis through antioxidants in culture medium. In 2015, Dione et al. established a quasi-universal culture medium for the cultivation of a large number of strains, including strict anaerobic bacteria (47). A culture medium, R-medium supplemented with antioxidants, namely, ascorbic acid, glutathione, and uric acid, allowed the growth of 251 different bacterial species. The preparation process of this medium is optimized for the culture of anaerobic species by preserving specific components. Specifically, components, including antioxidants, are sterilized by filtration at 0.22 μm to avoid the chemical reactions generated by autoclave sterilization (48). In fact, autoclave sterilization of agar in the presence of phosphate leads to the production of reactive oxygen species such as hydrogen peroxide that can inhibit the growth of anaerobic bacteria (48).

(ii) Targeting enzymatic activity to improve bacterial isolation. It is known that some bacterial species within a polymicrobial culture have a higher growth rate than others, occupying a large space and using a large number of nutrients. In this process, these phenomena inhibit the proliferation of slow-growing species. To solve this problem, Hou et al. used an inhibitor of the LpxC enzyme called CHIP-090 (49). The LpxC enzyme is a key enzyme in the biosynthesis of lipids in Gram-negative bacteria. Thus, this inhibitor has an antibacterial role against most Gram-negative bacteria (50), such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Bacteroides vulgatus* (49).

(iii) Use of specific micronutrients: tyrosine for targeting phenol- and *p*-cresol-producing bacteria. The specific culture of phenol- and *p*-cresol-producing bacteria was performed using rich media, as well as poor media (see Table 1) (51). Phenols are microbial metabolites produced from tyrosine. The supplementation of tyrosine and intermediary metabolites to basic media allowed the isolation of phenol- and/or *p*-cresol-producing strains (51). As a result, 26.3% of isolated species were phenol producers and 40.1% were p-cresol producers. Other bacterial metabolic pathways could be investigated in order to isolate species of interest targeted through their ability to metabolize specific micronutrients.

Innovative methods for isolating previously uncultured fastidious microorganisms. (i) Coculture. In continued efforts to grow uncultivated bacteria from environmental samples, previous studies have used other cultivated species to create optimal growth conditions using diffusion chambers (52–54) or double layers of agar separated using porous membranes (55). A coculture technique using a dependency test between slow- and fast-growing bacteria was used on different bacterial strains (56). "Helper-dependent" strains were identified as well as the pathway responsible for this phenomenon. This study showed that the presence of *E. coli* allowed the growth of several bacteria, such as *Faecalibacterium*, *Bilophila*, *Bacteroides*, *Suterella*, or *Gordonibacter*, via the menaquinone biosynthetic pathway, with menaquinones being one of the major classes of growth factors of species (56). Furthermore, this suggests that quinones can be added to some conventional culture media to promote the growth of bacterial symbionts. Another way of using symbiosis to grow uncultured bacteria *in vitro* was set up by Tanaka and Benno (57). This system consisted of soft agar layers separated by a 0.2-μm membrane with the upper or lower layer inoculated while warm and melting with single isolates from feces. The authors were able to mimic the mutualistic molecular interactions in the gut microbiome to isolate previously uncultured species (57).

(ii) Culture using reverse genomics. Recently, Cross et al. described an important breakthrough in the cultivation of previously uncultured microorganisms (10) and used a technique called reverse genomics, which combines genomics and flow cytometry with culture (Fig. 3). First, genes coding for membrane proteins from single-cell amplified genomes or metagenome-assembled genomes were identified. Then, the predicted target proteins with an extracellular domain were selected as epitopes. Specific antibodies were produced, purified, and labeled with a fluorochrome. Target cells were stained using the produced antibody, sorted, and subsequently sequenced and cultured. This method was used to sort *Saccharibacteria* from a human oral sample. The sorted fraction was cultured in different liquid media (BHI medium, OTEB medium, MTGE medium, and TSB medium supplemented with various additional factors) under anaerobic or hypoxic conditions. The reverse genomics method could be used for the targeted culture of other fastidious or previously uncultured microorganisms (58).

Contribution of culturomics to the study of the human microbiota. The contribution of culturomics to the current repertoire of bacterial species isolated from human samples since 2012 has been tremendous (3). Culturomics has drastically expanded the repertoire of the human microbiota mostly through the discovery of new bacterial species (8) in various niches, namely, the human gut, the skin, and urinary, vaginal, and respiratory tract microbiota (3, 8). To date, in our laboratory, we have been able to isolate nearly 800 new species (unpublished data). The supplementation of blood culture bottles with rumen and blood has been instrumental, as confirmed by another team that showed that the use of rumen and blood not only increased the number of isolates but also allowed the discovery of new bacterial species (19). Several studies also conducted by other teams have confirmed that the use of various conventional media, including selective media (BBE, BTB, CCFA, CIN, DHL, FM, Listeria, Mitis, MRS, Potato, Selenite, TM, and Tomato) and nonselective media (MH, BHI, CAN, PEA, GMM, Choco, Choco-pasteurized, and GAM [Table 1]) warranted by the concept of culturomics is very efficient to continuously advance the knowledge of human microbiome diversity (58).

Culturomics studies have established repeatedly their complementarity with metagenomics as shown in a recent study (59). The recent turning point in the study of the gut microbiota is thus geared toward a rebirth of culture-dependent methods. With each study, the dark matter of metagenomics and therefore the proportion of unassigned reads is reduced, supporting the crucial role of culturomics studies to characterize the human microbiota. Nevertheless, a new challenge is associated with this turning point toward high-throughput culture, the description of the numerous new species isolated. For that purpose, a polyphasic approach coined taxonogenomics combining phenotypic description with the genome sequence and the matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) spectrum was developed (60). Although a high number of new species were described using this concept (61–65), the process from isolation to publication of the description is quite long, taking up to 5 years to be officialized and available to the scientific community (66). New species announcements were created to allow prompt availability to the scientific community through the announcement of the isolation of a new species and its 16S rRNA gene and genome sequence and type strain deposition alongside basic phenotypic characteristics (66–76).

Tackling the culture of CPR: the next challenge in human microbiota culture. The Candidate Phyla Radiation (CPR) subdivision was first described in the environment at the turn of the century (77). Since then, CPR has been found in many ecosystems in the environment and more recently in the human microbiota (78). This supergroup covers over 15% of the bacterial domain in the tree of life, consisting of at least 35 phyla (79) that all share the characteristics of being very small organisms with very small genomes lacking basic biosynthetic abilities, such as the capacity to synthesize nucleotides and amino acids (80). These bacteria are episymbionts, thus fulfilling their biosynthetic deficits (80). The *Saccharibacteria* TM7 phylum was found to be a ubiquitous member of the oral cavity microbiota with a relative abundance of 1%. Very few isolates of this phylum have been cultured, mostly in coculture (10, 78, 81) and in rare instances in pure cultures (78, 81). Cross et al. used the aforementioned method of reverse genomics to coculture 7 phylotypes of TM7, all cocci with a diameter under 0.5 μm, among which two cocci in pure stable coculture with their host (10). Other studies have used other approaches to obtain CPR isolates. Through successive enrichment rounds in Fastidious Anaerobic Broth (FAB; LabM) supplemented with kanamycin (100 μg/ml) prior to plating on Fastidious Anaerobic Agar plates supplemented with 5% horse blood, Soro et al. were able to obtain a TM7 isolate from subgingival plaque in pure culture, forming creamy-white colonies with a diameter of 0.5 to 3 mm (78). This successive enrichment method was applied to saliva samples previously centrifuged at low power using SHI medium supplemented with increasing concentrations of streptomycin, from 100 to 500 μg/ml, in an anaerobic atmosphere and subsequently plated on SHI agar after each enrichment step. Stable cocultures of TM7x with its host bacterium *Actinomyces odontolyticus* strain XH001 were obtained after the third enrichment (SHI + 300 μg/ml of streptomycin) (81). Simpler approaches have also been successful, including filtration of the target samples; in this case, saliva is filtered at 0.2 μm to retain only ultrasmall particles, and subsequently the filtrate is cocultured with known oral commensals (82, 83). Although CPR has been detected in the gastrointestinal tract as well as in clinical samples, namely, blood (84), its proportion in these ecosystems is not well defined. Nevertheless, CPR has been associated with mucosal inflammation and inflammatory bowel disease (85). Tackling the challenge of routinely culturing CPR in pure isolates would be a tremendous step toward the ultimate goal, understanding the role of CPR in health and disease.

NEW STRATEGIES TO ISOLATE ARCHAEA FROM HUMAN MICROBIOTA

Background

In the last decade, the presence of archaea, as well as their importance for health, has been largely reported in the human microbiome due to the development of NGS and molecular methods such as PCR, qPCR, and FISH (5, 86, 87). Molecular methods

have described the taxonomic composition of the human archaeome as very site specific. The gastrointestinal tract and nasal cavity archaeomes are dominated by the *Euryarchaeota* phylum, while the cutaneous archaeome is dominated by the *Thaumarchaeota* phylum, and the lung archaeome mainly consists of the *Woesearchaeota* phylum (87, 88). Although archaea are increasingly detected in molecular data sets, very few human isolates have been isolated from the gastrointestinal tract (GIT) (89). This has created an avenue in microbiology with the end goal of allowing and simplifying the isolation of archaeal species from biological samples in order to explore their role in the homeostasis of the human body. In the last 5 years, there has been a tremendous advance in isolation techniques allowing the isolation of more archaeal strains from the GIT and other human niches.

Endogenous Production of Hydrogen: Coculture with Hydrogen Producers

Methanogenic archaea can be classified into three categories according to the type of substrate they are able to utilize to grow and produce methane. Hydrogenotrophic methanogens are able to reduce CO_2 using H_2, formate, or seldom secondary alcohols or ethanol as electron donors, while methylotrophic methanogens utilize methyl-group compounds to produce methane (90, 91). Finally, the third category consists of acetoclastic methanogens that are able to utilize acetate to grow (90). Standard archaeal culture thus requires an external input of a mixture of gas (and substrate) and therefore the necessary and expensive infrastructure to create, handle, and maintain the aforementioned atmosphere (91). Recently, through coculture with hydrogen-producing bacterial species, the need for an exogenous input of hydrogen was bypassed for the culture of hydrogenotrophic methanogens (92, 93). In fact, SCFA producers also produce gases such H_2 and CO_2 as by-products of fermentation. *Bacteroides thetaiotaomicron* was thus described as having a mutualistic relationship with *Methanobrevibacter smithii* (94, 95). This mutualistic relationship was utilized successfully to coculture *M. smithii* and *M. oralis* (96) with *B. thetaiotaomicron* (92, 96–100). *M. smithii* was also successfully cocultured in the presence of other hydrogen-producing bacteria, namely, *Bacteroides fragilis*, *B. vulgatus*, *Parabacteroides distasonis*, and *Desulfovibrio piger* (100). Species from the *Christensenella* genus have also been described as having a syntrophic relationship with *M. smithii*, and both were successfully cocultured (101). Enterobacteria can also be good candidates for coculture. In fact, *M. smithii* was isolated from urine samples only when a bacterial species was cultured from the same sample, mostly enterobacteria, which are known to produce hydrogen during the process of fermentation (93).

Coculture To Improve the Isolation of Archaeal Strains

The standard method of culturing archaea involves two steps that both require the input of hydrogen: an enrichment step, in the form of a liquid culture in Hungate tubes followed by seeding using the roll-tube method once methane is detected in the Hungate tube (102) in order to obtain colonies using a versatile medium permissive to the growth of methanogenic archaea (103). Coculture with hydrogen producers allows for the improvement of the enrichment step, as well as the seeding step through the double chamber system, which is a new, less fastidious, and less costly system (92). The need for external hydrogen can therefore be bypassed for the enrichment step. Subsequently, after methane detection, agar plates (SAB medium with antibiotics) are inoculated with the liquid culture and incubated in the upper compartment of a double chamber, while the lower compartment contained an aerobic culture of *B. thetaiotaomicron* in the presence of antioxidants (92). The culture of *B. thetaiotaomicron* thus provides the required external supply of hydrogen for *M. smithii* to grow. Colonies were identified using MALDI-TOF mass spectrometry (MS). This inventive system was improved by successfully coculturing *M. smithii* and a hydrogen-producing bacterium on solid medium in an ordinary anaerobic chamber (atmosphere consisting of a mixture of N_2 and CO_2) without any external supply of hydrogen (100).

Phylum	Species	GIT	Oral cavity	Urinary tract	Vagina	Breast milk/ colostrum	Infectious samples
Euryarchaeota	***Haloferax alexandrinus***	■					
	Haloferax massiliensis	■					
	Methanobrevibacter arboriphilicus	■					
	Methanobrevibacter millerae	■					
	Methanobrevibacter oralis	■	■			■	■
	Methanobrevibacter smithii	■	■	■	■	■	■
	Methanomassiliicoccus luminyensis	■					
	Methanosarcina mazeii		■				
	Methanosphaera stadtmanae	■	■				
	***Methanosphaera* sp. PA5**	■					

FIG 4 Repartition of archaea across human niches. Most isolated methanogenic archaea resided in the gastrointestinal tract (GIT). *Methanobrevibacter smithii* was the only species isolated from all human niches presented namely, the GIT, the oral cavity, the urinary tract and vagina, the breast milk/colostrum and infectious samples.

Archaea in the Human Microbiota and Implications in Health and Disease

Although the presence and isolation of archaeal species from the gastrointestinal tract (oral cavity and gut) have been described, the simplification of the culture technique of methanogens has allowed the isolation of archaeal strains from other human samples, such as urine (93), vagina (99), and milk and colostrum (96), therefore solidifying their place in the human microbiome. The improvement of culture techniques has allowed the isolation of a new member of the *Methanosphaera* genus (104) from human feces (Fig. 4). The introduction of halophilic culture of human feces has also allowed the first isolation of two members of the *Halobacteria* class from the GIT: *Haloferax alexandrinus* and a new species, *Haloferax massiliensis* (Fig. 4; see Table S1 in the supplemental material) (8, 105).

Very early implantation of *M. smithii* in the human GIT has been reported; this species was isolated from the gastric juice of newborns (97) and detected in the GIT using real-time PCR (97, 106). Furthermore, its absence early in life has been associated with severe acute malnutrition (107). Molecular methods have highlighted methanogens with several disorders in adults. Its reduction in adults was correlated with obesity, another type of malnutrition (108). The archaeome of inflammatory bowel disease patients is globally affected, with *M. smithii* taking the largest hit, and its balance shifted toward an increase in *Methanosphaera stadtmanae*, gearing the gut environment toward a more methylotrophic and therefore inflammatory setting (109). Conversely, *M. smithii* was increased in the vagina of bacterial vaginosis patients and could become a possible biomarker for this disease (110).

Although the isolation techniques of methanogens have greatly improved over the last 5 years, efforts to improve culture techniques and develop new techniques should be sustained. In fact, with *M. smithii* and other archaea being ubiquitous in the human microbiome across niches, it appears crucial to isolate other archaeal phyla, such as *Thaumarchaeota* and *Woesearchaeota*, from human samples in order to better explore the skin and lung archaeomes (87, 110) and multiply the number of isolates from different niches in the context of homeostasis and dysbiosis to investigate their implication in health and disease. Moreover, the availability of human isolates would allow the testing of archaea as probiotics in the context of severe acute malnutrition, for example, or as antibiotics as previously described from *Halobacteria* (111).

NEW STRATEGIES TO CULTURE EUKARYOTES FROM HUMAN MICROBIOTA

Background

Though not as studied as bacteria and archaea, eukaryotes represent a nonnegligible part of the human microbiota. Eukaryotes, including fungi, single-cell eukaryotes, namely, protists, and multicellular eukaryotes, such as helminths, have also been described in human niches (112, 113). Nonfungal eukaryotes were mostly described at first as parasites and/or pathogens but are increasingly described as commensals (114),

even in infants (115). Fungi are prevalent at a lower diversity than bacteria in the human microbiota and are detected across niches such as the gastrointestinal tract, skin, oral cavity, respiratory tract, and genitourinary tract (116–120). They are detected in infants as early as the first month of life and are probably transmitted to the infant from the vaginal mycobiota, the environment (121), and breast milk, the microbiota of which also contains fungi (122). Here, we chose to focus on the culture-dependent methods used to decipher the fungal diversity in the human microbiota.

Culture Conditions for Fungi in the Human Microbiota

Fungi and yeast are grown on liquid media and/or solid media usually containing antibiotics to inhibit bacterial growth (119). Liquid media such as Sabouraud dextrose broth or blood culture bottles (123) can be used to grow fungi or enrich samples before seeding on solid media (124). Classical solid media used for fungal isolation include Sabouraud dextrose agar, malt agar, potato dextrose agar, CZAPEK, Colombia agar, Dixon agar, modified Leeming Notman agar, YPD medium, and glycine-vancomycin-polymyxin B agar (see Table 1) (125–127). Some of these media, namely, Dixon agar and modified Leeming Notman agar, have the particularity to contain lipids and lipid derivatives to allow the growth of lipogenic fungi such as *Malassezia*. Chromogenic media have also been developed and are routinely used in clinical diagnosis, particularly for the diagnosis of urinary tract infections. These media exploit the enzymatic properties of different yeasts to identify several species through the specific color developed by the colony upon degradation of a specific substrate using a particular enzyme (128, 129). Most of these media share a characteristic, the presence of broad-spectrum antibiotics, which is crucial to inhibit bacterial growth, thus favoring fungal isolation. Chloramphenicol is widely used in these media, as well as, to a lesser extent, gentamicin, streptomycin, and cycloheximide (125).

The incubation temperature varies from 25°C for filamentous fungi to 37°C for yeast. The standard incubation time is 14 days with visual checking of the plates every few days (130). Nevertheless, filamentous fungi have been isolated after 4 days of isolation, and yeast, especially *Candida* species, can grow in less than 24 h (125, 131).

Evolution of Identification Methods over the Years

Classical identification methods use the principles of Pasteurian microbiology and are based on phenotypic characteristics determined by biochemical tests and microscopic observation after lactophenol staining (125). The advent of molecular methods improved fungal identification through sequencing of the 18S rRNA gene or the ITS regions, unveiling a wide diversity (122, 132). In the last decade, mass spectrometry has revolutionized the world of microbiology by exploiting the unique protein spectrum displayed by each species. It was first applied to bacterial identification and later extended to fungi. Conversely to bacteria, fungi require a protein extraction using ethanol, acetonitrile and formic acid prior to MALDI-TOF MS identification. MALDI-TOF MS technology has drastically reduced identification time and cost and allowed the exploration of a wide range of fungal communities (125, 133).

High-Throughput Culture: Culturomics Applied to Fungal Populations

Studies have used various media to isolate fungi from human samples at various incubation temperatures. Through the application of the principles of culturomics consisting of a variation of physicochemical parameters to explore the human mycobiota as exhaustively as possible, several culture conditions were used to explore the fungal diversity of a sample as exhaustively as possible. Twelve culture conditions were used in the most notable study, with supplementation with blood and rumen and plating on five culture media (Sabouraud agar, Dixon agar, potato dextrose agar, modified Schaedler agar, and banana agar medium) supplemented with three antibiotics (colistin, vancomycin, and imipenem) and incubation under aerobic and anaerobic conditions at 22, 28, and 42°C (123). The culturomics concept applied to fungal culture

combines MALDI-TOF MS and ITS sequencing to identify the high number of isolates in record time (123).

A Gap To Be Filled

Fungi and non-fungi eukaryotes have been shown to have an impact on immune responses and have also been linked to several diseases, as shown by a plethora of molecular studies (112, 134). As stated previously, though very informative, these methods do not provide isolates and therefore do not allow further experimentation to crossover to the application of the acquired knowledge. There are also reported cross-domain interactions in the human microbiota, warranting the need for isolates to provide a better understanding of these complex ecosystems (135). Nevertheless, since 1920 and the advent of culture techniques for fungi, the evolution of the aforementioned techniques has been quite limited. Efforts have been focused on yeasts, which are therefore more isolated and easily identified than filamentous fungi or mold. Anaerobic fungal isolates from the human gut also represent a glaring gap in knowledge, even though they have been described in the gut of herbivores (136). A shift of the research efforts is thus needed and should be geared toward an improvement of the isolation methods for the aforementioned fungi (136).

Strides have been made regarding identification techniques, all the way from Pasteurian methods to molecular methods and mass spectrometry (e.g., MALDI-TOF MS) (117, 127). Nevertheless, MALDI-TOF MS identification is still limited by the low number of referenced fungal spectra (125, 137). This low number is a reflection the number of isolates, emphasizing the need to improve fungal culture. The limitations in isolation and identification methods create a gap between culture-dependent and culture-independent methods (137). Culture-independent methods also face a glass ceiling, as databases are constantly incremented with new genome sequences. Therefore, a revival of culture-dependent methods to explore the human mycobiota is warranted.

BACTERIOTHERAPY APPLIED TO THE TREATMENT OF CERTAIN PATHOLOGIES

Cancer Therapy

Several notable studies (138) have highlighted beneficial commensals associated with response to cancer therapy (chemotherapy, immunotherapy). Species such as *Akkermansia muciniphila* (139), *F. prausnitzii*, *Phascolarctobacterium faecium* (140), *Bifidobacterium longum*, and *Collinsella aerofaciens* (141) have been associated with the response to immune checkpoint inhibitors in non-small-cell lung cancer/renal cell carcinoma and melanoma patients. These gut bacterial species impact the response to chemotherapy and immunotherapy by eliciting a beneficial response of the immune system in mouse models (138). Some species are able to increase cytotoxic immune populations. For example, in mouse models, *Enterococcus hirae* and *Barnesiella intestinihominis* have shown the ability to positively modulate the immune response to cyclophosphamide (CTX). Upon injection of CTX, *E. hirae* was able to increase the intratumoral CD8/Treg ratio by translocating to secondary lymphoid organs, whereas post-CTX, *B. intestinihominis* was able to promote intratumoral IFN-γ/$\gamma\delta$T cells, both resulting in reduced tumor size (142). *A. muciniphila*, found to be increased in the gut microbiome of non-small-cell lung cancer and renal cell carcinoma patients responsive to anti-PD1, was able to reverse the nonresponder to anti-PD1 phenotype in "avatar" mice by inducing the recruitment of intratumoral $CCR9^+$ $CXCR3^+$ $CD4^+$ T lymphocytes in mouse models (139). *F. prausnitzii* and *P. faecium* improved systemic and antitumor immune responses due to increased antigen presentation and enhanced function of effector T cells in the periphery and microenvironment of the tumor in melanoma patients (140).

Other species positively modulate the immune response through the production of immunostimulatory cytokines. For example, *Alistipes shahii* led to an increase in the production of tumor necrosis factor alpha by intratumoral myeloid cells in response to immunotherapy consisting of anti-interleukin-10 (anti-IL-10) and cytosine phosphate-

guanosine oligodeoxynucleotides (CpG-ODN) in mouse models (143). *B. thetaiotaomicron* and *B. fragilis* led to tumor regression in response to anti-CTLA-4 through dendritic cell maturation and modulated IL-12-dependent TH 1 responses in tumor-draining lymph nodes (144). Both species have been shown to reduce anti-CTLA-4-associated toxicity in mice and are more prevalent in patients resistant to anti-CTLA-4-associated colitis in humans (145). A synergy between *Bifidobacterium* species and anti-PD1 led to tumor regression in mice by increasing the maturation of dendritic cells by upregulating MHC-II presentation, thus enhancing cytotoxic T cell priming in the tumor microenvironment (146).

Metabolic Disease Therapy

Some gut commensal species have been associated with metabolic homeostasis and thus might be protective against metabolic disorders (147). *A. muciniphila* is a mucin-producing, short-chain fatty acid (SCFA)-producing bacterium that is highly prevalent in the human gut and actively participates in gut barrier function (148). Several mouse studies have shown that *A. muciniphila* is underrepresented in overweight mice or in the context of obesity or type 2 diabetes (149). Moreover, *A. muciniphila* was inversely correlated with the increase in markers of inflammation, lipid synthesis or insulin resistance (149, 150). Supplementation of *A. muciniphila* in the gut of mice by oral gavage led to a decrease in weight and fat and an improvement in intestinal barrier functions in these mice, improved glucose tolerance and decreased endogenous hepatic glucose production (149, 151). Another group of SCFA producers, the *Christensenellaceae* family, was shown to be lacking in people with obesity (136) and upregulated in women with obesity after a weight-loss diet (152). Supplementation of *Christensenella minuta*, a member of this family, in the gut of mice with obesity causes a decrease in fat gain (153), and an antibiotic-mediated decrease in *C. minuta* gut prevalence increases the risk of obesity in mice (154).

Inflammatory Disease Therapy

In patients with chronic inflammatory bowel disease (IBD), the diversity and composition of the intestinal microbiota are consistently altered. *F. prausnitzii*, one of the most abundant bacteria of the human intestinal microbiota with a proportion of approximately 5% of the total fecal bacteria, has been shown to induce anti-inflammatory effects (155). Many studies have shown that *F. prausnitzii* and *A. muciniphila* were decreased in patients with IBD (156–158). In fact, IBD patients present reduced barrier function. Supplementation of the gut microbiota of mice with induced colitis with *F. prausnitzii* led to a marked attenuation of colitis, weight loss, a decrease in proinflammatory cytokines, and an increase in anti-inflammatory cytokines (156). The shift in the balance toward anti-inflammatory cytokines was also caused by enrichment of *A. muciniphila* in the gut of mice (159).

PERSPECTIVES

The last few years have seen a turning point in the study of the human microbiota with a regained interest in culture strategies. The evolution of technology as well as the better understanding of microbial metabolism achieved mostly through NGS have allowed great improvements in the isolation of previously uncultured species. Efforts in this field in the last 5 years have resulted in the isolation of species of interest as well as more than 700 previously uncultured species by several teams. Nevertheless, some challenges still need to be addressed, including the culture of CPR from habitats other than the oral cavity. The availability of all of these isolates opens perspectives in the field of bacteriotherapy. In fact, fecal microbiota transplantation is becoming the standard to treat diseases such as *C. difficile*-induced diarrhea or multidrug-resistant bacterial infection. With the increase in the availability of strains of interest, bacteriotherapy can represent a viable and wide-range alternative to FMT and, as such, become a part of the future of medicine.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

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